2969-Pos Board B124

Mutual Macromolecular Crowding in Polymer Solutions

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Ogston and Laurent conceived and established a theory for steric exclusion of spherical particles such as globular proteins by a suspension of randomly oriented fibers. In their model, a protein can just touch the surface of a fiber, so the space occupied by the fiber itself, in addition to a cylindrical shell with a thickness equal to the radius of the protein, are spaces excluded to the protein. In a suspension of many fibers, the effective concentration of the protein is increased, because the available volume is reduced. This model was successfully applied to explain a number of properties (association and conformational equilibria, partition between compartments, osmotic pressure, etc.) for solutions of proteins in the presence of flexible polymers such as hyaluronan (HA), and the relevance of steric exclusion to the properties of biological tissues was established. Because small globular proteins are excluded only from a cylindrical shell immediately surrounding a linear HA chain, the crowding effect is sensitive to the mass concentration of the polymer segments, but not the molecular mass of the polymer chain. Matsuoka and Cowman subsequently developed a semi-empirical expression to quantitatively account for the concentration, shape, and molecular mass dependence of physicochemical properties (steady shear viscosity, osmotic pressure, light scattering, etc.) of HA or other polymer solutions. A connection between the Ogston-Laurent and Matsuoka-Cowman approaches will be presented, providing a firm foundation for understanding polymer solution properties on the basis of mutual macromolecular crowding, where steric exclusion depends on the effective hydrodynamic volume of the polymer. This theory can be employed to explain the dependence of amyloid protein fibril formation on the molecular mass of crowding polymers.

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The Usage of Proteolitic Enzymes Inhibitors in Studies of the Blood Plasma Particle Size Distribution by the Dynamic Light Scattering

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Dynamic light scattering (DLS) can be used to obtain the particle size distribution in various human liquids (e.g. saliva, blood samples - plasma, serum, etc.) in order to carry out diagnostic of some diseases. However the effect of proteolytic enzymes on the peptides compounds causes to a continuously changing of particle size distribution with time after blood donation. To stabilize the size distribution pattern the proteolytic enzymes inhibitors should be added to the blood samples.

The aims of this study are to obtain the particle size distribution in blood plasma with adding of proteolytic enzyme inhibitors solution (Sigma-Aldrich No. P2714) and in the absence of it by methods of DLS.

Two samples of blood were collected from each of healthy non-smoking volunteers (n=6): one sample was with the proteolytic enzymes inhibitors and another without it. Fresh plasma was prepared from each blood sample by centrifugation through 3-4 hours after donation.

The results were obtained using an experimental set-up for DLS consisting of a laser beam (633 nm) and a Digital Real-Time Correlator ''PhotocorFC''. The DynaLS code provided results graphically in the form of scattering light intensity distribution on particles sizes.

Particle size distributions for each sample were obtained through 6 hours after its donation. These distributions were proved to significantly differ at the big sizes region. The particles had significantly larger diameters in samples with adding enzymes inhibitors (996 - 1985 nm) than in the samples without it (492 - 785 nm). We believe that these results could be an evidence of particles splitting processes slow down. Subsequent measurements through 9 hours after donation confirmed the slowing of decay of the biggest particles of blood plasma.

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Understanding the Kinetics of Membrane Recruitment of Rac GTPases using Single Particle Tracking

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Rho family of small GTPases are involved in majority of cellular processes, from cell growth, to cell migration, cell division etc. Of these proteins, the Rac GTPases are key regulators of cytoskeletal dynamics and remodeling of actin. Besides cycling between a GTP-bound and a GDP-bound state, Rac also undergoes a membrane targeting cycle. The precise steps of activation and/or membrane-trafficking of Rac are still the subject of a lot of speculations. To address the in-vivo transition kinetics of Rac GTPases from inactive cytosolic state to membrane-associated state, we employed total internal reflection (TIR) microscopy and single particle tracking methods. Epithelial MCF-7 cells and constructs expressing wtRac1 GTPase fused with photoconvertible fluorophore, mEos3, were used for the study. From displacement histogram analysis, we identified two populations of membrane-bound Rac 1 GTPases - a highly mobile population and a relatively stationary population. Also, further analysis with Hidden Markov Model (HMM) revealed that the molecules may exist in different mobility states and undergo state-switching during their membrane lifetime. The fast diffusive state with Diffusion coefficient of 0.28μm2/sec possibly corresponds to freely-moving GDP-bound Rac. The slow diffusing state, with a 10-fold lower diffusion coefficient might indicate some interaction with GTP Exchange Factor (GEF). The diffusion of Rac GTPases also showed a differential pattern in the leading edge/lamellipodia under stimulation with EGF or under conditions of constitutive Rac expression with higher percentage of active GTP-bound molecules. These differences might be explained by the activated versus the inactivated states of the membrane targeted Rac GTPases.

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Determination of Quaternary Structure of Rhodopsin at Room and Body Temperature using Spectral FRET

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Rhodopsin is a prototypical G-protein coupled receptor that initiates phototransduction in the retina of the eye. Like many other G protein-coupled receptors, rhodopsin appears to form oligomers in the membrane. The notion that rhodopsin forms oligomers in its native membrane, however, is still quite controversial. The clearest demonstration that rhodopsin forms oligomers comes from atomic force microscopy images displaying large arrays of rhodopsin monomers. It has been speculated, however, that oligomers observed in these images are due to phase separation occurring in membranes at temperatures below 37 C. In the work presented here, we explored in detail the oligomeric status of rhodopsin and its dependency on temperatures using spectrally resolved Resonance Energy Transfer (RET) in Chinese hamster ovary cells. As a control, we also investigated a G188R mutant rhodopsin. This mutation causes misfolding of rhodopsin and leads to the retinal degenerative disorder retinitis pigmentosa. Our results suggest that the quaternary structure of wild-type rhodopsin is vastly different compared to that of the misfolded mutant rhodopsin.

2973-Pos Board B128

Computational Prediction of ALS Patient Survival Times from Protein Mechanical Properties

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Superoxide dismutase-1 (SOD1) is a Cu and Zn binding, homodimeric free radical defense enzyme, whose misfolding and aggregation play a key role in amyotrophic lateral sclerosis (ALS), a presently incurable and invariably fatal neurodegenerative disease. Over 150 mutations in SOD1 have been identified with a familial form of the disease, but it is presently not clear what unifying features, if any, these mutants share to make them pathogenic. We have developed a new computational assay to answer this question. We probe the mechanical properties of ALS-associated SOD1 mutants by simulating a series of atomic force microscopy experiments with variable tether positions. Such assays would be currently difficult or impossible experimentally, but by harnessing the power of computer simulations to manipulate proteins in a virtual environment, mechanical force studies may be designed to directly address those processes critical to the propagation of misfolding and its role in disease. These studies enabled us to quantify a mechanical rigidity ''fingerprint'' characterizing a given SOD1 variant, and as well to measure the severity of a given mutation upon structural integrity, metal affinity, and dimer stability. All ALS-associated mutants studied showed reduced structural integrity, an increased tendency to lose either Cu or Zn, and an increased tendency to monomerize; such processes are suspected to be critical in the progression of ALS. Upon closer analysis, we found that these stability and metal affinity measurements showed a remarkable ability to predict the lifetime of an ALS patient once neurodegenerative symptoms have been diagnosed.